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RELATIONSHIP BETWEEN THE MORPHOLOGY OF *CLAVICEPS PURPUREA* AND THE PRODUCTION OF ALKALOIDS

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(With Plates 34 and 35 and 1 Text-figure)

The production of alkaloids by certain strains of *Claviceps purpurea* (Fr.) Tul. has been correlated with the morphological form of constituent hyphae, both in surface and submerged culture and in the sclerotia produced on host plants.

In saprophytic culture, strains of *Claviceps purpurea* (Fr.) Tul., isolated from natural sclerotia or conidia, produce mainly unorganized mycelium and conidia. It has been observed that such strains do not produce alkaloids in culture under any of the conditions which have been tested. In previous investigations with *Claviceps paspali* Stevens & Hall (Tonolo, Scotti & Vero-Barcellona, 1961), a correlation was established between the morphology of the mycelium in submerged culture and the production of alkaloids.

Two strains of *C. purpurea* have been isolated which produced alkaloids in culture, and this paper describes a comparative study of the morphology of the mycelium obtained in culture and the formation of sclerotia on plants, both with reference to the production of alkaloids.

MATERIALS AND METHODS

Origin of Claviceps purpurea *isolates*

Isolates were made from two separate samples of ergot sclerotia. The first was collected from rye at Bries, N. Italy, in 1960, and the isolate was designated F-91. The second was collected from a hybrid between wheat and rye (*Triticale*) (Pl. 35, fig. 7) at Porrino, Spain, in 1961, and was designated F-1317/3. Analysis of the remaining sclerotia of the Spanish sample showed the alkaloid content to be 0·17 %, consisting mainly of ergotamine and some ergotoxine. This value may have been a little higher initially as the analysis was made several years after harvest.

Culture medium and conditions

The following medium (modified from Stoll, Brack, Hofmann & Kobel 1957) was used throughout these studies, whether in liquid culture (100 ml medium per 500 ml Erlenmeyer flask) or as agar slopes: sucrose 100 g, asparagine 10 g, $Ca(NO_3)_2$ 1 g, $MgSO_4.7H_2O$ 0·25 g, KH_2PO_4 0·25 g, KCl 0·125 g, $FeSO_4.7H_2O$ 0·033 g, $ZnSO_4.7H_2O$ 0·027 g, yeast extract 0·1 g, cysteine hydrochloride 0·01 g, distilled water 1 l, pH 5·0.

Liquid cultures were grown at 24 °C, either stationary for 20 days or in continuous shake culture on a rotary shaker (200 rev/min and 10 cm eccentric throw) for 8–10 days.

Analytical

Quantitative estimations of alkaloid in culture filtrates were made colorimetrically after direct reaction with the reagent of Ehrlich and van Urk (0·125 % *p*-dimethylaminobenzaldehyde in 65 % H_2SO_4 + 0·1 % of 5 % $FeCl_3$). Qualitative determination of constituent alkaloids was made by a combination of paper and thin-layer chromatographic techniques. (Arcamone *et al.* 1961; McLaughlin, Goyan & Paul, 1964). Colorimetric and chromatographic analysis of alkaloids from ergots was made after exhaustively extracting the finely ground sclerotia with ether and shaking out the alkaloids from the ether extract with 1 % tartaric acid.

Host plants

Seeds of Svalofs Fourex rye and a Russian *Triticale* hybrid were kindly supplied by Dr A. Hagberg (Swedish Seed Association, Svalof) and two varieties of wheat (Thew and Fylgia) were kindly supplied by Prof. A. J. P. Oort, Wageningen, The Netherlands.

Host plant florets were inoculated with a suspension of spores, or hyphal fragments if the strain was non-sporing, after ear emergence and before anthesis, using a hypodermic syringe.

RESULTS

Morphology of natural ergot sclerotia

The sclerotia of *C. purpurea* are the resting stage in the life cycle of the fungus and normally give rise to the sexual stage. The sclerotia consist of a plectenchymatic tissue formed by polygonal cells, 10–15 μ in diameter, containing fat droplets and alkaloids (Text-fig. 1 A). In this paper, the use of the term *plectenchymatic* refers to a fungal tissue formed by polygonal cells which are derived from filamentous hyphae, but having lost the filamentous arrangement (Text-fig. 1 B). The term *prosenchymatic* refers to a fungal tissue built of closely packed, thickened hyphae retaining a clear pattern of filamentous arrangement (Text-fig. 1 C) (Ainsworth, 1963).

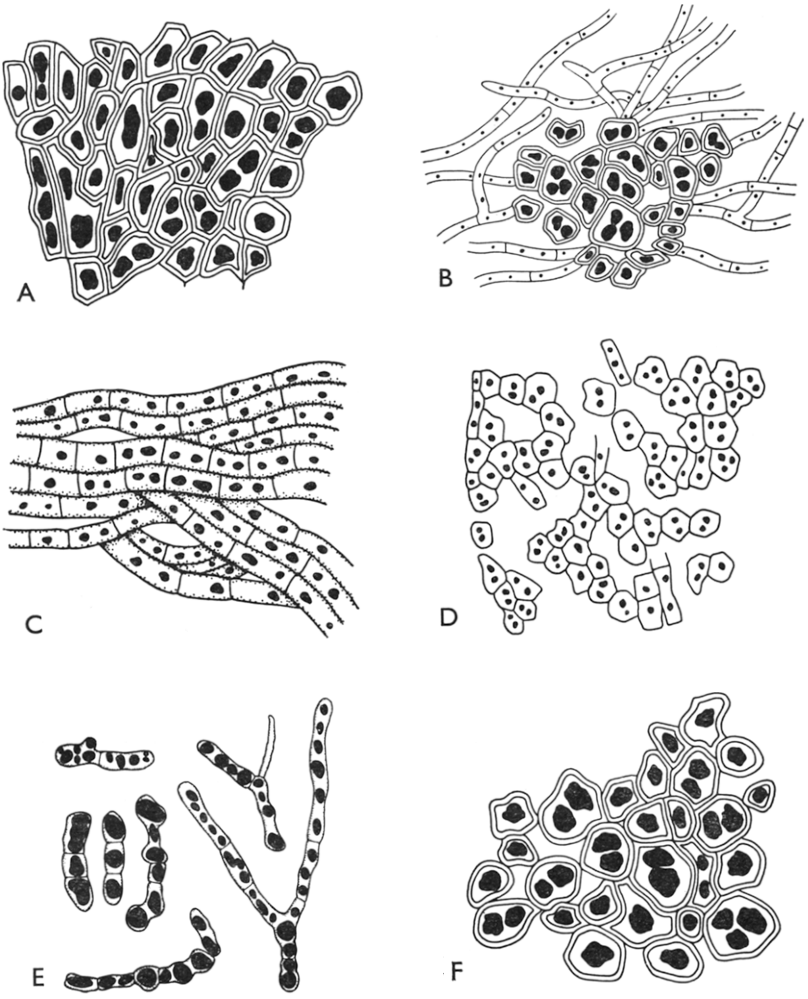
Morphology of Claviceps purpurea *strains on agar*

Strain F-91 was not homogenous, and on the sucrose-asparagine agar separated into two distinct morphological types which were designated F-91/s and F-91/c.

Strain F-91/s (Pl. 34, figs. 1, 2) produced colonies at the centre of which the mycelium formed raised cushions (5–8 mm diam) of plectenchymatic tissue (Pl. 34, fig. 4), having a red-violet colour and bearing droplets which contained many conidia (Pl. 34, fig. 3).

Strain 91/c consistently produced flat white colonies which also produced many conidia. No plectenchymatic or prosenchymatic tissue was present.

Strain 91/s constantly showed a tendency to change morphologically to the form of 91/c, but strain 91/c never produced a 91/s form. Thus it



Text-fig. 1. (A) T.S. typical sclerotium of *C. purpurea* showing plectenchymatic tissue. (B) Section of a colony of strain F-1317/3/11 on agar showing the origin of plectenchymatic tissue by thickening of the hyphae. (C) Prosenchymatic tissue constituting part of the atypical sclerotia of strain F-1317/3/11 (Pl. 35, fig. 10). (D) Section of a plectenchymatic pellet of strain F-1317/3/11 from submerged culture (Pl. 34, fig. 6). (E) Hyphae from submerged culture of strain F-1978 composed of short thick 'sclerotial' cells. (F) Plectenchymatic tissue from agar culture of strain F-1978. (All × 500. Dark areas represent fat content).

was necessary to maintain the characteristic plectenchymatic form of 91/s by repeated selection from plectenchymatic tissue of this strain.

Strain F-1317/3 was quite different from both 91/s and 91/c. On sucrose–asparagine agar the colonies were off-white and consisted of a compact white plectenchymatic tissue. No conidia were produced.

Morphology of strains and production of alkaloids in surface culture

Strain 91/s produced, in sucrose–asparagine broth, a thick, pigmented, plectenchymatic mat. The culture filtrate contained up to 150 μg alkaloids/ml., mainly ergotamine, and similar alkaloid was also present within the hyphae. By comparison, strain 91/c formed a thin white mycelial mat bearing many conidia, and neither mycelium nor culture filtrate contained alkaloids.

In the same medium, strain F-1317/3 produced a thick plectenchymatic mat and both mycelium and culture filtrate contained alkaloids, mainly ergotamine. The yield of the original strain was about 20 μg alkaloids/ml, which was increased to about 300 μg/ml by repeated selection (strains F-1317/3/11 and F-1978). These strains differed significantly from strain 91/s since they did not produce conidia.

Morphology of strains and alkaloid production in submerged culture

Strain 91/s consistently produced filamentous mycelium in all media tested, and no alkaloid was detected. The hyphae were thin, 2–3 μ wide, containing few fat droplets, and conidia were present.

Strain 91/c behaved similarly.

By comparison, strains F-1317/3/11 and F-1978 produced alkaloids (100 and 300 μg/ml respectively) and this was correlated with the formation either of plectenchymatic pellets (Pl. 34, figs. 5, 6; Text-fig. 1 D) or of short thick hyphal filaments, both of which, in structure, form and fat content, resembled a sclerotial tissue (Text-fig. 1 E).

Relationship between sclerotial formation and alkaloid content on host plants

In saprophytic culture there appeared to be a clear correlation between alkaloid production and the sclerotial form of growth. In order to extend this correlation, inflorescences of host plants were inoculated with the various strains. Conidial inocula of strain 91/s consistently produced typical ergot sclerotia (Pl. 35, fig. 11) on all the tested host plants, after producing the normal sphacelial state. The alkaloid content varied between 0·15 and 0·53 %, according to the host plant, and consisted solely of ergotamine. By comparison, strain 91/c did not proceed beyond the sphacelial state and only formed soft, white bodies which were confined to the floral cavity and contained no detectable alkaloids (Pl. 35, fig. 11). Anatomically, this tissue was neither plectenchymatic nor prosenchymatic but consisted solely of loose hyphae and many associated conidia. This rather unusual observation was made in three replicated experiments. When the fungus was re-isolated from either the typical ergots of 91/s

Table 1. *Summary of morphological characters and alkaloid production capacity of variants of two isolates of* Claviceps purpurea *grown in vitro and in vivo*

C. purpurea strains		F-91/s	F-91/c	F-1317/3	F-1317/3/11	F-1978
Alkaloid production in surface culture (μg/ml)		150	0	20	100	300
Alkaloid production in submerged culture (μg/ml)		0	0	0	100	300
Morphology in surface culture		Plectenchymatic sclerotial mat and conidia	Conidia and mycelium	Plectenchymatic sclerotial mat No conidia		
Morphology in submerged culture		Loose mycelium and conidia	Loose mycelium and conidia	Loose mycelium only	Spherical pellets with plectenchymatic sclerotial structure	Spherical pellets with plectenchymatic sclerotial structure, or sclerotial hyphae
Characteristics of sclerotia on host plants	Morphology	Typical plectenchymatic ergot sclerotia	Sphacelial stage only	Morphologically atypical sclerotia, with plectenchymatic and prosenchymatic tissue in random arrangement		Typical plectenchymatic ergot sclerotia
	Alkaloid content	0·2%	0·0%	0·03%	0·1%	0·3%

or the sphacelial tissue of 91/c the growth forms characteristic of these strains were again obtained.

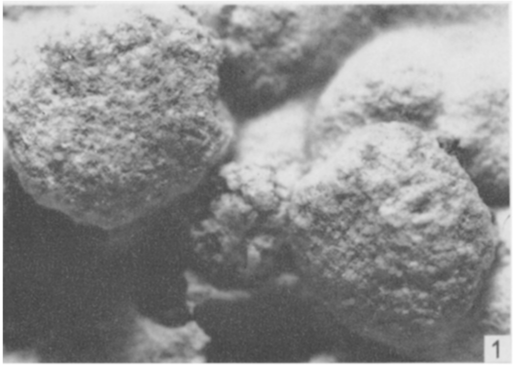
Host plants were inoculated with hyphal fragments of strains F-1317/3, F-1317/3/11, and F-1978. The sphacelial state was abnormal in that where honeydew was exuded from the florets it was mostly free from conidia. The sclerotia obtained from strains F-1317/3 and F-1317/3/11 were atypical (Pl. 35, figs. 9, 10). Morphologically the sclerotia were roughly spherical, white, and consisted of an apparently random mixture of plectenchymatic and prosenchymatic tissues. The alkaloid content varied between 0·03 and 0·10 % and consisted mainly of ergotamine. However, typical ergot sclerotia (Pl. 35, fig. 8) were obtained from Strain F-1978 and had an alkaloid content of 0·24–0·32 % which also consisted mainly of ergotamine.

DISCUSSION

In these experiments (summarized in Table 1) a clear correlation was observed between the morphology of mycelium and alkaloid production when strains F-91/s and F-91/c were grown in saprophytic culture. A parallel correlation was also obtained when these strains were established on host plants. Further, in the series of re-selections derived from strain F-1317/3, a clear correlation was observed between increasing alkaloid production in saprophytic culture and the change from atypical to typical sclerotia, after these strains had become established on host plants. It is not clear why the original isolate, F-1317/3, failed consistently to produce typical sclerotia on rye, wheat and various grasses and even on *Triticale* hybrids from which the strain was originally isolated. However, this strain was unusual in not producing conidia, whereas it has been found that all the other freely sporulating isolates which have been tested have always produced typical sclerotia when inoculated into the host florets.

The formation of a plectenchymatic tissue in saprophytic culture by strains of *C. purpurea*, described in this paper, associated with alkaloid production, does not imply that this tissue is physiologically identical with natural ergot sclerotia with regard to the ability subsequently to produce the sexual state.

The significance of obtaining a specific morphological form of growth or association of fungal cells for the in vitro production of a particular metabolite has already been recorded (Dion, Carilli, Sermonti & Chain, 1954; Testi-Camposano, 1959), but it is believed that the studies described in this paper show for the first time that this correlation may extend also into the parasitic role of a plant pathogen. Further, it is suggested that in attempts to obtain and improve alkaloid yields of *Claviceps purpurea* in vitro an important aspect to be borne in mind is the production of a plectenchymatic growth form.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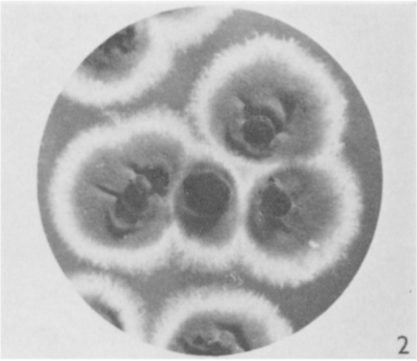
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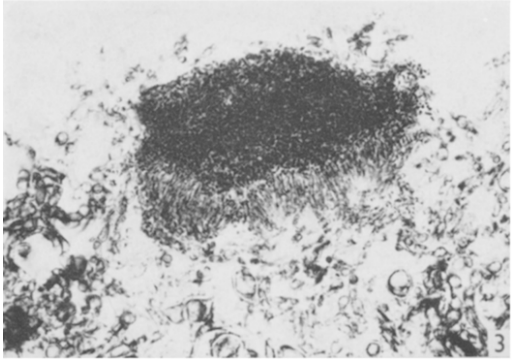
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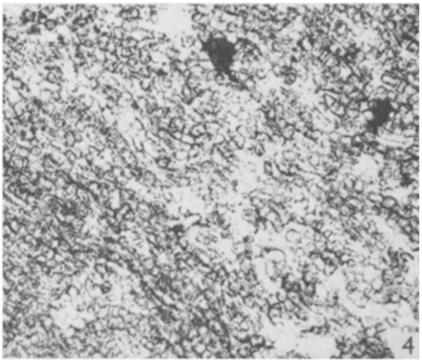
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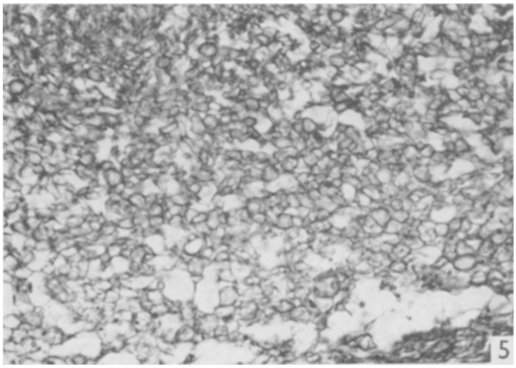
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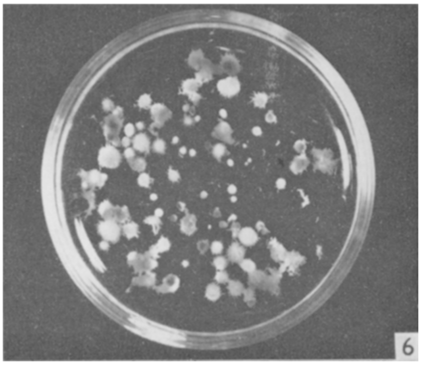
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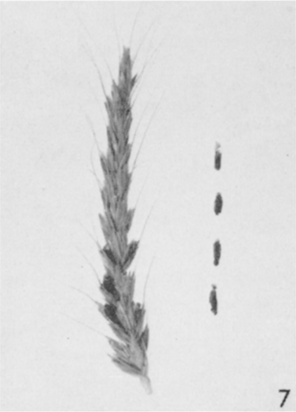

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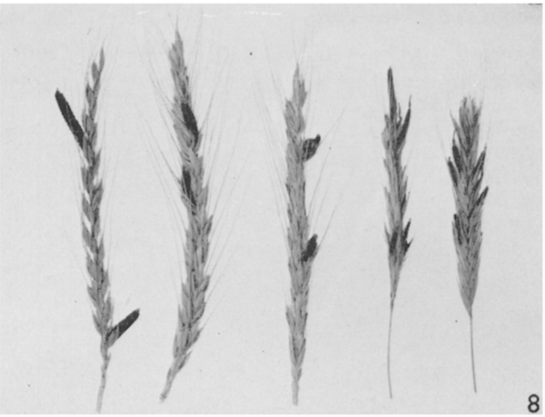

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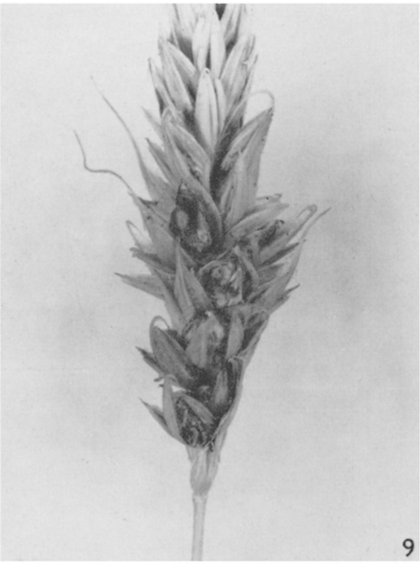

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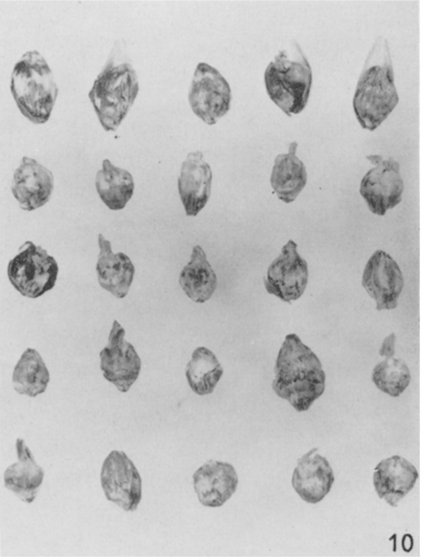

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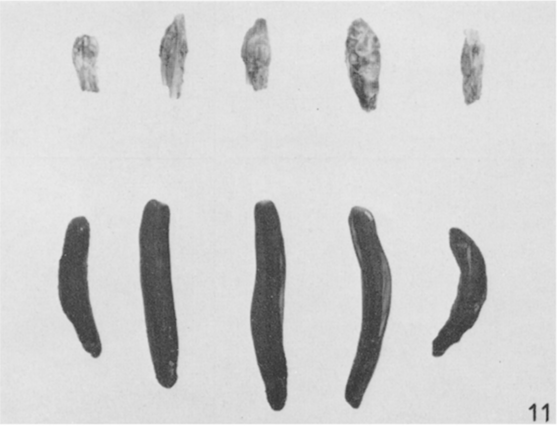

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EXPLANATION OF PLATES 34 and 35

PLATE 34

Fig. 1. Detail of 'sclerotial' cushions of strain 91/s on agar (fig. 2) showing sphacelial droplets (×4·4).

Fig. 2. Colonies of strain 91/s on agar showing pigmented central area, with raised cushion, and white periphery. (×0·87).

Fig. 3. V.S. sclerotial cushion of strain 91/s on agar (fig. 1) showing discrete sphacelial fructification amongst loose hyphae on the upper surface of the cushion (×350).

Fig. 4. Plectenchymatic 'sclerotial' formation of constituent hyphae at the centre of a 'sclerotial' cushion of strain 91/s (fig. 1). (×350).

Fig. 5. Plectenchymatic central tissue of a pellet of strain F-1317/3/11 from submerged culture (fig. 6) (×350).

Fig. 6. Pellets from submerged culture of strain F-1317/3/11 (×0·44).

PLATE 35

Fig. 7. Original Spanish *Triticale* hybrid bearing sclerotia from which strain F-1317/3 was isolated (×0·53).

Fig. 8. Left, three *Triticale* ears, and right, two rye ears, each bearing typical sclerotia of strain F-1978 (×0·35).

Fig. 9. *Triticale* hybrid bearing atypical sclerotia of strain F-1317/3/11 (×1·3).

Fig. 10. Detail of fig. 9 (×1·3).

Fig. 11. Above, atypical sphacelial structures of strain 91/c developed on rye; below, typical ergot sclerotia of strain 91/s developed on rye (×1·3).

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